L27: Entry 2 of 6 File: USPT Dec 17, 2002

DOCUMENT-IDENTIFIER: US 6495363 B2
TITLE: In-line complete spectral fluorescent imaging of nucleic acid molecules

Brief Summary Text (12):
The GBA.TM. Genetic Bit Analysis method disclosed by Goelet et al. (WO 92/15712, herein incorporated by reference in its entirety) is a particularly useful microsequencing method. In GBA.TM., the nucleotide sequence information surrounding a predetermined site of interrogation is used to design an oligonucleotide primer that is complementary to the region immediately adjacent to, but not including, the predetermined site. The target DNA template is selected from the biological sample and hybridized to the interrogating primer. This primer is extended by a single labeled dideoxynucleotide using DNA polymerase in the presence of at least two, and most preferably all four chain terminating nucleoside triphosphate precursors.

Brief Summary Text (13):
Mundy (U.S. Pat. No. 4,656,127, herein incorporated by reference in its entirety) discusses alternative microsequencing methods for determining the identity of the nucleotide present at a particular polymorphic site. Mundy's method employs a specialized exonuclease-resistant nucleotide derivative. A primer complementary to the allelic sequence immediately 3'-to the polymorphic site is permitted to hybridize to a target molecule obtained from a particular animal or human. If the polymorphic site on the target molecule contains a nucleotide that is complementary to the particular exonucleotide-resistant nucleotide derivative present, then that derivative will be incorporated by a polymerase onto the end of the hybridized primer. Such incorporation renders the primer resistant to exonuclease, and thereby permits its detection. Since the identity of the exonucleotide-resistant derivative of the sample is known, a finding that the primer has become resistant to exonucleases reveals that the nucleotide present in the polymorphic site of the target molecule was complementary to that of the nucleotide derivative used in the reaction. Mundy's method has the advantage that it does not require the determination of large amounts of extraneous sequence data. It has the disadvantages of destroying the amplified target sequences, and unmodified primer and of being extremely sensitive to the rate of polymerase incorporation of the specific exonuclease-resistant nucleotide being used.

Brief Summary Text (15):
In contrast to the method of Cohen et al. (French Patent 2,650,840; PCT Appln. No. WO91/02087), the GBA.TM. method of Goelet et al. can be conducted as a heterogeneous phase assay, in which the primer or the target molecule is immobilized to a solid phase. It is thus easier to perform, and more accurate than the method discussed by Cohen. The method of Cohen has the significant disadvantage of being a solution-based extension method that uses labeled dideoxynucleoside triphosphates. In

Brief Summary Text (21):
Boyce-Jacino et al. have described a method for sequencing a polynucleotide using nested GBA (U.S. patent application Ser. No. 08/616,906, herein incorporated by reference in its entirety). In that method, an array of nested primer oligonucleotides is immobilized to a solid support. A target nucleic molecule is hybridized to the array of nested primer oligonucleotides and the hybridized array is sequenced using GBA.

Brief Summary Text (33):
Bogdanov et al. disclose the fluorescent imaging and quantification of solid support-bound nucleic acids (Bogdanov et al., SPIE Proceeding 2985:129-137 (1997), herein incorporated by reference in its entirety). The Bogdanov et al. reference discloses direct multicolor fluorescent imaging of a GBA.TM. array (GBA.TM. microchip) on a solid-state support with low background emission (glass microscope slide) for simultaneous (CCD camera) and sequential (commercial FluorImagers) reaction spots reading at excitation of various lasers. Bogdanov et al. employ a filter-based confocal optics configuration. Two-color fluorescent images of oligonucleotides labeled by fluorescein and CY3 were reported, as was CCD-based imaging of a direct multispot GBA.TM. image extension detection reaction using fluorescein-labeled ddATP.

Brief Summary Text (36):
The present invention provides a hyperspectral fluorescent imaging apparatus for microarray detection which comprises: (a) a light source, wherein the light source is capable of emitting a transmission beam; (b) an expansion lens; (c) a focusing lens, wherein the focusing lens focuses said transmission beam into a thin focus line; (d) a collection lens; (e) an imaging spectrometer; and (f) a detector.

Brief Summary Text (37):
The hyperspectral fluorescent imaging apparatus of the present invention may further comprise a translation stage.

Detailed Description Text (3):
The present invention provides both a hyperspectral fluorescent imaging apparatus and methods for employing such an apparatus for multi-dye/base detection on a microarray. More specifically, the present invention describes a multi-color, solid-phase, hyperspectral (complete spectrum) imaging apparatus and methods thereof which enables highly sensitive, rapid and low-cost analysis of primer extension arrays. The present invention further provides a rapid and cost-effective fluorescent detection apparatus and methods thereof with the capability of spectrally discriminating four dye labels on a high density DNA microarray. The method of the present invention has general applicability to the analysis of multi-color arrays in other tests, such as hybridization or differential display. Under one embodiment, the present invention can be used to detect a mutation in a gene that, for example, plays a causative role in diseases such as cancer (p53 and BRCA2 are two examples of such genes).

Detailed Description Text (14):
Alternatively, a photomultiplier ("PMT") with related optics modification may be used in the present invention. However, a CCD detector remains the preferred embodiment over a PMT in microchip fluorescent detection because of the CCD's compatibility with the 2-D microchip's metrics. As used herein, the phrase 2-D microchip's metrics means that the microchip is a plane detection object. In other words, the metrics of the detected object (microarray) is 2 dimensional. Detection efficiency is maximized when the object and detector metrics are matched. With a CCD, it is not necessary to scan the microarray point-by-point, as in a PMT, and a CCD allows for parallel fluorescent imaging of the entire DNA chip.

Detailed Description Text (19):
Miniaturized oligonucleotide arrays for mutation detection present a strategy for overcoming the problems associated with gel-based methods (DeRisi et al., Nature Genetics 14:457-460 (1996), herein incorporated by reference in its entirety; Hacia et al., Nature Genetics 14:441-447 (1996), herein incorporated by reference in its entirety; Head et al., Nucleic Acids Res. 25:5065-5071 (1997)). The present invention offers significant advantages over gel-based sequencing methods in several areas, including sample processing simplicity, through-put and reagent cost. These advantages are realized because microarray technology allows for highly parallel analysis of samples with minimal reagent usage and purification steps. For example, a 10 .mu.l multiplexed PCR reaction can be hybridized simultaneously to hundreds or thousands of oligonucleotides in an array only a few millimeters in diameter. In this way, processing is performed on a "macro" scale, using standard pipettes, with the information being extracted on a "micro" scale, using fluorescent imaging.

Detailed Description Text (32):
The sequencing reagents of the present invention are intended to be made into an array. As used herein, the phrase "sequencing reagent" is intended to refer to a reagent which is capable of being extended in a polymerase-mediated, template-dependent fashion. Accordingly, the phrase is intended to encompass DNA, RNA and PNA sequences or combinations thereof. The sequencing reagents of the present invention can be either synthetically or naturally made. As used herein, a natural sequencing reagent includes, but is not limited to, such reagents as a gene or fragment thereof, a cDNA molecule or fragment thereof, and an EST molecule or fragment thereof. As used herein, an array is an orderly arrangement of sequencing reagents, as in a matrix of rows and columns or spatially addressable or separable arrangement such as with coated beads. Preferably, the array is an array of nested sequencing reagents. As used herein, a nested array is an array of reagents whose sequence specific hybridization regions sequentially overlap in sequence. By using an array of nested sequencing reagents, it is possible to determine the sequence of the target nucleic acid.

Detailed Description Text (71):
Fluorescent Imaging of High-density Arrays

Detailed Description Text (72):
An entire image of a microchip array (1.times.1 cm.sup.2 area) is captured by using a standard cooled CCD (1000.times.1000 pixel format, 10.mu. pixel size) with 1.times.1 amplification optics. In this case, the spatial resolution of the detection system is equal to the CCD pixel size (=10.mu.). This spatial resolution is sufficient for the detection of a GBA array with a density of greater than 10,000 spots/cm.sup.2. Resolution and sensitivity of CCD fluorescent imaging for a DNA microchip is illustrated by FIG. 5 which demonstrate that the CCD detector is capable of detecting 10.sup.-18 M of fluorescently labeled oligo molecules with a resolution of at least 70.mu..

Detailed Description Text (95):
After extension, SPS signals are immediately detected with the fluorescent imaging method of the present invention. The quantification of the SPS reaction can done using a software program, (e.g., MTI Image) and base/dye calling results are generated.

Other Reference Publication (2):
Bogfanov et al., "Fluorescent imaging and quantitation of solid support bound nucleic acids", SPIE Proceeding, 2985:129-137 (1997).

CLAIMS:

1. A hyperspectral fluorescent imaging apparatus for microarray detection which

comprises: (a) a light source, wherein the light source is capable of emitting a transmission beam for hyperspectral fluorescent imaging; (b) an expansion lens that expands the transmission beam for microarray detection; (c) a focusing lens that focuses the expanded transmission beam into a thin focus line for microarray detection; (d) a translation stage; (e) a collection lens that collects fluorescent light emitted at the thin focus line; an imaging spectrometer for hyperspectral fluorescent imaging; and (g) a detector.

2. A hyperspectral fluorescent imaging apparatus for microarray detection which comprises: (a) a light source, wherein the light source is capable of emitting a transmission beam for hyperspectral fluorescent imaging; (b) a first expansion lens and a second expansion lens that expand the transmission beam for microarray detection; (c) a focusing lens that focuses the expanded transmission beam into a thin focus line for microarray detection; (d) a collection lens that collects fluorescent light emitted at the thin focus line; (e) an imaging spectrometer for hyperspectral fluorescent imaging; and (f) a detector.

3. A hyperspectral fluorescent imaging apparatus according to claim 1, wherein the detector is a charge coupled device.

4. A hyperspectral fluorescent imaging apparatus according to claim 1, wherein the light source is a laser.

5. A hyperspectral fluorescent imaging apparatus according to claim 4, wherein the laser is a CW Ar laser.

6. A hyperspectral fluorescent imaging apparatus according to claim 1, wherein the apparatus comprises a first expansion lens and a second expansion lens.

7. A hyperspectral fluorescent imaging apparatus according to claim 2, wherein the apparatus further comprises a translation stage.

8. A hyperspectral fluorescent imaging apparatus according to claim 2, wherein the detector is charge coupled device.

9. A hyperspectral fluorescent imaging apparatus according to claim 2, wherein the light source is a laser.

10. A hyperspectral fluorescent imaging apparatus according to claim 9, wherein the laser is a CW Ar laser.

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(FILE 'HOME' ENTERED AT 15:30:23 ON 11 MAR 2004)

FILE 'STNGUIDE' ENTERED AT 15:30:33 ON 11 MAR 2004

FILE 'HOME' ENTERED AT 15:30:37 ON 11 MAR 2004

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT' ENTERED AT 15:30:49 ON 11 MAR 2004

L1 4 S DENSHAM D?/AU

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 15:31:41 ON 11 MAR 2004

L2 139850 S (TARGET OR POLYNUCLEOTIDE) AND (ENZYME OR HELICASE OR PRIMASE
L3 90 S L2 AND (ENZYME (5A) CONFORMATION? CHANGE)
L4 1 S L3 AND ((DETERMIN? OR DETECT? OR IDENTIFY?) (2A) SEQUENC?)
L5 0 S L3 AND ((FRET (2A) PAIR) OR (ACCEPTOR AND DONOR))
L6 7 S L3 AND LABEL?
L7 393 S L2 AND ((FRET (3A) PAIR) OR (ACCEPTOR AND DONOR) OR (ACCEPTO
L8 247 S L7 AND SEQUENC?
L9 61 S L8 AND (SOID SUPPORT OR SUPPORT OR ARRAY)
L10 2 S L9 AND (PRIMASE OR HELICASE)
L11 51 DUP REM L9 (10 DUPLICATES REMOVED)
L12 20 S L9 AND (TARGET (3A) (POLYNUCLEOTIDE OR NUCLEIC ACID OR DNA O
L13 15 DUP REM L12 (5 DUPLICATES REMOVED)

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Determination of intraparticle immobilized enzyme distribution in porous support by confocal scanning microscopy

AUTHOR(S): Pinto, M. C.; Macias, P.
CORPORATE SOURCE: Fac. Ciencias, Univ. Extremadura, Badajoz, 06071, Spain
SOURCE: Biotechnology Techniques (1995), 9(7), 481-6
CODEN: BTECE6; ISSN: 0951-208X
PUBLISHER: Science and Technology Letters
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A method for determining the distribution of immobilized protein within a porous support\ has been developed. The method is based on confocal microscopy of polyacrylamide gel **beads** coupled to fluorescein isothiocyanate labeled enzyme. It is applied to immobilized soybean lipoxygenase. This technique allows the quant. and qual. anal. of protein distribution profile in intact polymer **beads** without splitting or cutting the carrier.

Interaction of chloroquine with linear and supercoiled DNAs. Effect on the torsional dynamics, rigidity, and twist energy parameter.

AU Wu P G; Song L; Clendenning J B; Fujimoto B S; Benight A S; Schurr J M
CS Department of Chemistry, University of Washington, Seattle 98195.
NC R01-GM29338 (NIGMS)
SO Biochemistry, (1988 Oct 18) 27 (21) 8128-44.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198905
ED Entered STN: 19900306
Last Updated on STN: 19970203
Entered Medline: 19890502
AB The magnitude and uniformity of the torsion elastic constant (alpha) of linear pBR322 DNA and supercoiled pBR322 DNAs with high-twist (sigma = -0.083) and normal-twist (sigma = -0.48) are measured in 0.1 M NaCl as a function of added chloroquine/base-pair ratio (chl/bp) by studying the fluorescence **polarization anisotrophy** (FPA) of intercalated ethidium dye. The time-resolved FPA is measured by using a picosecond dye laser for excitation and time-correlated single-photon counting detection. A general theory is developed for the binding of ligands that unwind superhelical DNAs, and the simultaneous binding of two different intercalators is treated in detail. The equilibrium constant (K) for binding chloroquine to linear pBR322 DNA and the number (r) of bound chloroquines per base pair are determined from the relative amplitude ratio of the slow (normally intercalated) and fast (free) components in the decay of the (probe) ethidium fluorescence intensity as a function of chl/bp. For chloroquine binding to supercoiled pBR322 DNAs, the intrinsic binding constant is assumed to be the same as for the linear DNA, but the twist energy parameter ET (N times the free energy to change the linking number from 0 to 1 in units of kBT) is regarded as adjustable. Using the best-fit ET, the binding ratios r are calculated for each chl/bp ratio. Twist energy parameters are also determined for ethidium binding to these supercoiled DNAs by competitive dialysis. For chloroquine binding, we obtain ET = 360 and 460 respectively for the normal-twist and high-twist supercoiled DNAs. For ethidium binding the corresponding values are ET = 280 +/- 70 and 347 +/- 50. Like other dye-binding values, these are substantially lower than those obtained by ligation methods. In the absence of chloroquine, the torsion constants of all three DNAs are virtually identical, alpha = (5.0 +/- 0.4) x 10(-12) dyn.cm. For linear pBR322 DNA, the magnitude and uniformity of alpha remain unaltered by intercalated chloroquine up to r = 0.19. This finding argues that the FPA is not significantly relaxed by diffusion of any kinks or solitons. If alpha d denotes the torsion constant between a dye and a base pair and alpha 0 that between two base pairs, then our data imply that alpha d/alpha 0 lies in the range 0.65-1.64, with a most probable value of 1.0.(ABSTRACT TRUNCATED AT 400 WORDS)
CT Check Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.
*Chloroquine: PD, pharmacology
*DNA: DE, drug effects
*DNA, Superhelical: DE, drug effects
Mathematics
Models, Theoretical
*Nucleic Acid Conformation
Plasmids: DE, drug effects
Stress, Mechanical
Thermodynamics
RN 54-05-7 (Chloroquine); 9007-49-2 (DNA)
CN 0 (DNA, Superhelical); 0 (Plasmids)

Detection of low copy numbers of HPV DNA by fluorescent in situ hybridization combined with confocal microscopy as an alternative to in situ polymerase chain reaction.

AUTHOR(S): Lizard, G. [Reprint author]; Chignol, M.-C.; Souchier, C.; Roignot, P.; Chardonnet, Y.; Schmitt, D.

CORPORATE SOURCE: INSERM U498, CHU/Hopital Bocage, Lab. Biochimie Med., BP 1542, 21034 Dijon Cedex, France

SOURCE: Journal of Virological Methods, (May, 1998) Vol. 72, No. 1, pp. 15-25. print.
CODEN: JVMEDH. ISSN: 0166-0934.

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LANGUAGE: English

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Last Updated on STN: 13 Aug 1998

AB In genital lesions infected by human papillomavirus (HPV), histological criteria and HPV DNA typing are of prognostic value. Therefore, non-radioactive methods such as in situ hybridization are used extensively since they preserve the histological organization of the tissue, and allow the detection and characterization of HPV DNA. However, the sensitivity of these methods is often limited to detection of low copy numbers of HPV DNA in isolated cells or in tissue sections, and therefore alternative techniques have been explored. In the present study, 1-2 copies of HPV DNA were visualized in SiHa cells either by in situ amplification of **nucleic acid** sequences with the polymerase chain reaction (PCR) or by fluorescent in situ hybridization (FISH) associated with observation by laser scanning confocal microscopy (LSCM). The latter procedure was evaluated for use on histological tissue sections to identify low copy numbers of HPV DNA. Genital lesions which were negative by enzymatic in situ hybridization and FISH but histologically suspected of HPV infection were investigated, and intense signals were obtained both with in situ PCR and with the combined use of FISH and LSCM. Therefore, the combination of FISH with LSCM examination may be as valuable as in situ PCR to detect viral genes present in small amounts in isolated cells and in tissue sections.

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